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14. ABSTRACT
The Epithelial-to-Mesenchymal Transition (EMT) is a conserved developmental process that is thought to be reactivated during the metastasis of epithelial cancers such as breast cancer. This study seeks to identify genes commonly regulated in the EMT, and identify key regulators of the process. From the successful candidate regulators, a core genetic circuit that controls the EMT will be constructed. The relevance of this circuit, or portions of it, to cancer metastasis, particularly in breast cancer models, will be investigated. An EMT core gene signature of approximately 1000 genes was generated, from which the transcription factor Zeb1 emerged as a potential key regulator of the EMT. Zeb1 demonstrated a much stronger EMT-inducing ability than previously known factors. The relationship between known EMT-inducing factors has not previously been studied, so this represents a novel finding. Efforts are underway to construct the genetic circuit stemming from Zeb1.

15. SUBJECT TERMS
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Introduction

Breast cancer is one of the most common forms of cancer for women in the developed world[1]. Mortality is a result not of the primary tumor, but of complications arising from metastases that have spread beyond the breast tissue itself. The study of breast cancer progression and metastasis is therefore almost as important as the study of the initiation of the cancer itself. The Epithelial-to-Mesenchymal Transition (EMT) is a conserved embryonic developmental process where epithelial germ layer cells acquire the properties of mesenchymal cells, allowing the cells to migrate away from their original site[2]. The EMT is thought to be reactivated during cancer metastasis, and may facilitate the dissemination of cancer cells to distant organs. The EMT is not well-defined at a molecular level, and it is not known which aspects of the EMT may be important in cancer metastasis. This study seeks to identify genes that behave consistently upon EMT, regardless of the initial triggering event. That information will be used to identify candidate regulators of the EMT. Successful candidates will serve as anchor points to molecularly define the core genetic circuit that controls the EMT. The eventual hope, beyond the scope of this study, is to use this information to guide the development of measures that can inhibit the process of EMT, and along with it, metastatic progression.

Body

With regard to the training plan, all tasks are being completed according to schedule as detailed in the Statement of Work. All meetings, symposia and conferences listed in the Statement of Work were attended. The teaching component of the training plan was begun in February 2009 and will be completed in May 2009.

With regard to the research plan:

1. Generate a core gene expression signature of the EMT using the HMLE-derived cell lines

Using cell lines already available in the lab, there were no technical difficulties in obtaining the raw materials needed to perform the microarray experiment to generate gene expression profiles. HMLE cell lines constitutively expressing Snail, Twist, Goosecoid, TGF-β1 and short hairpin RNA against E-cadherin were used, along with the relevant control lines. The microarray experiment was performed at the Broad Institute, which has ample experience and expertise in the technique. Raw data post-processing was also handled at the Broad Institute, and the result was a statistically significant core signature of approximately 1000 genes which are consistently up- or down-regulated after the cells have converted from an epithelial to a mesenchymal phenotype.

2. Use the core gene signature to find key components of the EMT regulatory circuitry

Attempts to draw a gene network solely from the microarray data were unsuccessful. Several common bioinformatics procedures were attempted and did not yield strong candidates[3,4]. Correlating gene expression to genomic locations commonly amplified or deleted in cancers did not produce result. Comparing existing cancer metastasis gene signatures to the core signature did not yield any overlap over what would be expected by chance[5]. Finally, comparison of the core signature to existing high quality microarray data from clinical samples of melanoma primary tumor versus melanoma metastases produced a significant overlap[6]. Further analysis of the promoters of the overlapping set of genes[7] resulted in the identification of the transcription factor Zeb1 as a likely mediator of many genes whose activities change upon the EMT.

3. Test the effect and importance of candidate key components on the EMT in HMLE and other cells in vitro

It was found that Zeb1 constitutively expressed in HMLE cells was able to induce the EMT. Furthermore, it was able to do so on a much shorter timescale than other known EMT-inducing factors. The relationship between Zeb1 and other known EMT-inducers is currently being investigated. The studies will reveal whether Zeb1 requires the other EMT-inducers to be present to induce or maintain the EMT, and *vice versa*. The testing of the ability of Zeb1 to induce the EMT in other cell types is underway and on schedule. The building of a regulatory network using Zeb1 as an anchor point is behind schedule. There have been problems in obtaining a good Zeb1 antibody, which is required to generate good quality whole-genome Chromatin Immunoprecipitation-on-chip (ChIP-on-chip) data. There is also a delay in creating an inducible Zeb1 expression system which is required for kinetic studies to identify the genes up- or down-regulated immediately upon Zeb1 activity. Priority is currently given to creating the inducible Zeb1 expression system, either through a Zeb1-estrogen receptor fusion protein or a doxycycline-inducible Zeb1 expression construct. It is believed that this route has a greater chance of success and is able to generate more useful data.

4. Demonstrate that key components of the EMT can profoundly influence the ability of tumorigenic cells to metastasize in vivo

Not yet underway.

5. Correlate the activity of key components of the EMT with metastasis of human breast cancers and other cancers, using laser capture microdissected tumor samples

Not yet underway.

6. Develop a usable signature of EMT that can be used as a predictor of a primary tumor's tendency to metastasize

Not yet underway.

Key Research Accomplishments

- Generated EMT core gene signature from microarray data
- Identified Zeb1 as a key candidate transcription factor mediating the EMT
- Demonstrated Zeb1 induces the EMT more quickly than previously known factors

Reportable Outcomes

- EMT core gene signature
- Human Mammary Epithelial Cell lines expressing Zeb1 and relevant control lines
- Human Mammary Epithelial Cell lines expressing short hairpin RNA targeting Zeb1 and relevant control lines

Conclusion

The results obtained so far provide encouragement that the objectives of the training grant are achievable. The identification of Zeb1 is not in itself novel, as other recent work has also demonstrated that Zeb1 can mediate the EMT[8-11]. However, most groups studying the EMT do not consider the interaction between the known EMT-inducers, and only study them separately. By studying them in concert, this study can potentially reveal a hierarchical order, with some factors closer to the decision to trigger the EMT than others. The novel finding that Zeb1 can trigger the EMT more quickly than other known factors suggests that Zeb1 may occupy a more prominent position in the hierarchy, and makes it highly desirable to explore Zeb1's connection to other genes, both downstream and upstream. The gene network arising from such explorations may constitute a core EMT regulatory circuit, applicable not only to the model system being used, but other types of epithelial tissue malignancies.

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